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Review

The emerging role of epigenetics in rheumatic diseases

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Abstract

Epigenetics is a key mechanism regulating the expression of genes. There are three main and interrelated mechanisms: DNA methylation, post-translational modification of histone proteins and non-coding RNA. Gene activation is generally associated with lower levels of DNA methylation in promoters and with distinct histone marks such as acetylation of amino acids in histones. Unlike the genetic code, the epigenome is altered by endogenous (e.g. hormonal) and environmental (e.g. diet, exercise) factors and changes with age. Recent evidence implicates epigenetic mechanisms in the pathogenesis of common rheumatic disease, including RA, OA, SLE and scleroderma. Epigenetic drift has been implicated in age-related changes in the immune system that result in the development of a pro-inflammatory status termed inflammageing, potentially increasing the risk of age-related conditions such as polymyalgia rheumatica. Therapeutic targeting of the epigenome has shown promise in animal models of rheumatic diseases. Rapid advances in computational biology and DNA sequencing technology will lead to a more comprehensive understanding of the roles of epigenetics in the pathogenesis of common rheumatic diseases.

Key words: epigenetics, DNA methylation, rheumatic diseases, histone code, histone deacetylases, environment, ageing, therapeutic targeting.

REVIEW

Introduction

Although most cells have the same DNA sequence, the activity of individual genes differs significantly between different cell types and tissues, e.g. the insulin gene is highly compacted in structure and inactive in all tissues except pancreatic β cells, in which it is in an open conformation that facilitates transcription. Epigenetics has been defined as heritable changes in gene expression that are not encoded by the DNA sequence itself [1]. Unlike the latter, it is dynamic and changes under the influence of endogenous and environmental factors. The role of epigenetics in health and disease is emerging; it has been established that carcinogenesis is frequently associated with epigenetic alterations that are potential therapeutic targeting [2, 3]. Our objectives are to give overviews of the epigenetic mechanisms regulating gene expression, their involvement in the pathogenesis of common musculoskeletal conditions and their potential as therapeutic targets.

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Epigenetic mechanisms

Three major interrelated mechanisms regulate gene expression: DNA methylation, post-translational modification of histone proteins and non-coding RNA (ncRNA).

DNA methylation

In plants, yeasts and animals, methylation of DNA mainly occurs at the cytosine (C) residues of DNA in CpG dinucleotide motifs and is regulated by DNA methyltransferase (DNMT) enzymes. Overall, CpG motifs are predominantly methylated, except in CpG-rich regions of 200–300 bp in the 5′ region of many genes, termed CpG islands, which are mainly unmethylated. In general the regions around the transcriptional start site of active genes have low levels of methylation [4]; conversely, gene bodies are methylated [5]. In macrophages, for example, methylation of CpG motifs in the TNF promoter within 200 bp of the transcriptional start site is low, at ~20%, while upstream CpG motifs have levels >80% [6]; similar findings have been described in IL-6 [7]. Greater variability exists in DNA methylation levels between different tissues from an individual than in identical

tissues from different individuals [8]. The existence of DNA demethylating enzymes is generally accepted, but the molecular mechanisms are poorly understood and are believed to involve the enzyme ten-eleven translocation 1 (TET1) [9]. Demethylation is an important mechanism governing gene activation; the IL-2 promoter demethylates within 20 min of activation of T cells [10], facilitating binding of the transcription factor Oct-1 [11].

Epigenetics also regulates alternative splicing [12] and promoter usage [5], processes that add significantly to the complexity of the expressed genome. Furthermore, in diploid cells ~5% of genes are monoallelically expressed [13]; in lymphoblastoid cells this affects a disproportionately high number of cell surface proteins and cytokines (IL-2, −4, −5 and −13) [14, 15]. It seems likely that this phenomenon is mediated by allele-specific methylation [16, 17]. Genetic variants influence the variability of DNA methylation levels between individuals, however, the heritability of this trait has not been determined [18].

Histone code

More than 100 post-translational modifications of the N-terminal tails of histone proteins have been described, including acetylation, methylation, phosphorylation and sumoylation [19]. These result in changes in the structure of nucleosomes, altering access of the transcriptional machinery and transcriptional activity (Fig. 1). The histone code is linked with DNA methylation by distinct, but coupled, pathways [20]. Histone acetylation is considered a permissive transcriptional mark, while trimethylation is associated with repression [21, 22]. Histone acetylation and phosphorylation are rapidly modifiable, while methylation is more stable; the terminal transferase gene (*Dntt*) undergoes silencing during thymocyte maturation characterized by initial deacetylation of H3-Lys9 and subsequent methylation at H3-Lys9, the latter being irreversible [23]. The deacetylation at H3-Lys9 begins in the promoter region (500 kb on either side of the transcriptional start site) and then spreads across the *Dntt* locus (22 kb) at a rate of 2 kb/h. Deacetylation is reversible, but the subsequent methylation at H3-Lys4 is not and results in permanent silencing of *Dntt*.

Non-coding RNAs

The coding exons of genes comprise ~1.5% of the human genome. It has recently become clear from the Encyclopedia of DNA Elements (ENCODE) project that a large proportion of the genome codes for non-protein coding RNA species that have important roles in regulating the transcriptome [24]. ncRNAs are primarily classified according to size: short (20–60 bp), mid-size (60–200 bp) and long (>200 bp). There is a large body of evidence implicating disrupted expression of ncRNAs in neoplastic and inflammatory diseases [25]. MicroRNAs (miRNAs) are short ncRNA molecules that regulate gene expression mainly by targeting the cognate RNA molecule for degradation or translational inhibition. In addition, several miRNAs alter gene expression by binding to complementary sequences in gene promoters with resultant alterations in the histone signature [26]. There is significant cross-regulation of the three epigenetic mechanisms: expression of many miRNAs are modulated by DNA methylation and histone modifications (reviewed in [27]), and miRNAs have been shown to target key proteins regulating the epigenome; expression of DNMT3A and DNMT3B are modulated by miR-29 [28], and HDAC1 and HDAC4 are targeted by miR-449a [29] and miR-1 [30], respectively.

Epigenetic influences in RA

Synovial fibroblasts

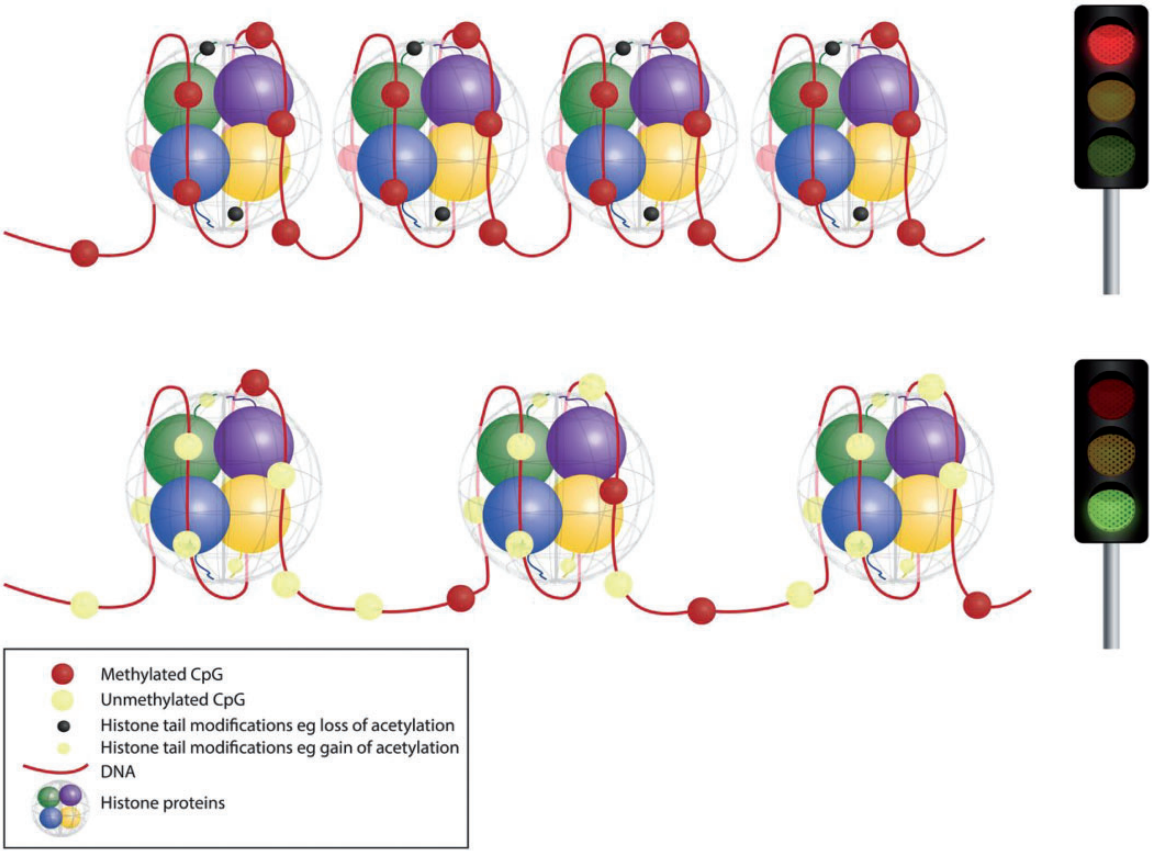
The RA fibroblast-like synoviocytes (RASFs) are central mediators of tissue destruction via the production of a range of disease-related molecules, including chemokines, adhesion molecules and proteases [31]. In addition, RASFs have a semi-transformed phenotype *in vitro*, with loss of contact inhibition, high proliferative activity and resistance to apoptosis. Engraftment of normal human cartilage and RASFs into the severe combined immunodeficiency mouse revealed this aggressive phenotype to be maintained for up to 60 days and be independent of adaptive immune cells [32, 33]. The mechanism(s) responsible for this stable, aggressive phenotype is unknown, however, there is increasing evidence implicating the epigenome. A methylation array study reported lower levels in RASFs compared with OA synovial fibroblasts (OASFs), particularly in genes regulating cell adhesion, transendothelial migration and extracellular matrix interactions [34]. Furthermore, treating OASFs with the DNA demethylating agent 5-azadeoxycytidine (AZA) resulted in conversion to an RASF-like phenotype [35]. Decreased expression of miR-34a*, as a result of increased promoter methylation, results in up-regulation of the X-linked inhibitor of apoptosis protein, potentially contributing to the resistance of RASFs to apoptosis [36].

The acetylation of histone proteins is regulated by the relative activities of two enzyme families: histone acetyltransferases and histone deacetylases (HDACs). The HDAC superfamily is important in the regulation of a wide range of developmental and physiological processes [37]. As HDACs lack DNA-binding activity, they are recruited to target genes via interactions with transcription factors. A shift towards histone hyperacetylation has been reported in RASFs compared with OASFs [38, 39], with overexpression of HDAC1 in the former [39]. Targeted knockdown of HDAC1 in RASFs, using small interfering RNA, resulted in decreased proliferation and increased apoptosis [40].

Peripheral blood

Global DNA methylation has been reported to be lower in T cells [41] and leucocytes [42] of RA patients compared with controls, although both involved small numbers. A single CpG motif in the IL-6 promoter, ~1 kb upstream of the transcriptional start site, was significantly less methylated in peripheral blood mononuclear cells from RA cases compared with controls, and correlated with higher lipopolysaccharide-induced IL-6 mRNA levels by

Fig. 1 Epigenetic modifications control the transcriptional status of genes.



In the upper panel DNA methylation (red) and chemical changes in histone tails, such as lysine acetylation, resulted in a closed chromatin structure and resultant repression of transcription. Conversely, DNA demethylation (light yellow) and histone modifications, such as lysine deacetylation, resulted in an open chromatin structure and transcriptional activation.

monocyte-derived macrophages [7]. Higher expression of CD40L and lower promoter methylation is found in RA CD4 T cells [43].

Epigenetic influences in OA

The underlying pathogenic mechanisms of OA are poorly understood but involve genetic and environmental factors. Studies of the role of the epigenome have concentrated on chondrocytes. Genomic DNA methylation levels were found to be similar in chondrocytes from 10 OA and 10 normal joints [44], however, the levels of methylation of the leptin promoter were lower in chondrocytes isolated from severely involved cartilage compared with minimally involved or normal cartilage and were associated with greater expression of this catabolic cytokine and its downstream target MMP (MMP-3) [45]; similar findings have been reported in MMP-9, MMP-13 and ADAMTS (a disintegrin and metalloproteinase with a thrombospondin type 1 motif) [46]. Of particular note is the finding of lower ADAMTS-4 promoter methylation and higher expression in lesional compared with non-lesional chondrocytes [47]. Nitric oxide (NO), a key signalling molecule, is produced at high levels by activated chondrocytes [48] and mediates IL-1β-induced suppression of cartilage proteoglycan synthesis [49]. Lesional chondrocytes express high levels of inducible NO synthesis (iNOS) and have reduced methylation of a nuclear factor κB (NF-κB) enhancer element 5.8 kb upstream of the iNOS transcriptional start site [50]. These studies reveal the importance of comparing the epigenetic profiles of chondrocytes from lesional and non-lesional cartilage within the same OA joint.

Epigenetic influences in SLE

Alterations in the epigenome have been implicated in both idiopathic and drug-induced lupus. Lower levels of genomic DNA methylation have been reported in peripheral T cells from lupus patients compared with healthy controls [41], and adoptive transfer of T cells that have been treated with AZA induces a lupus-like condition in syngeneic mice [51]. Incubation of human T cells with this agent results in alterations in gene expression similar to those found in idiopathic lupus, including the up-regulation of CD11a [52]. An additional epigenetic abnormality in lupus T cells is the reduced expression of DNMT1 secondary to decreased

activity of Ras-mitogen-activated protein kinase [53]. It is notable that the drugs associated with the development of iatrogenic lupus, procainamide and hydralazine, have been shown to be functional inhibitors of DNMT, potentially resulting in DNA hypomethylation [54, 55].

SSc

A central feature of SSc is tissue fibrosis mediated by interstitial fibroblasts. These cells have an altered phenotype, both *in vivo* and *in vitro*, characterized by excessive deposition of extracellular matrix proteins including collagens [56, 57]. The maintenance of this phenotype *in vitro* has been correlated with higher levels of DNMT1 [57, 58] and treatment with DNA demethylating agents *in vitro* results in reduced expression of collagen by dermal fibroblasts from SSc patients, but has no effect on fibroblasts from healthy controls [57]. A major suppressor of collagen transcription is FLi1, which is down-regulated in SSc dermal fibroblasts [59]. This alteration is associated with higher methylation of a CpG island in the FLi1 promoter [57]. These data suggest that higher DNA methylation is present in SSc dermal fibroblasts and may be an important mechanism governing the overproduction of collagen. Expression of miR-29, which targets collagen gene transcripts, is lower in SSc dermal fibroblasts compared with fibroblasts from controls, and overexpression in the former resulted in decreased collagen gene expression [60].

The epigenome as a therapeutic target

The pathways regulating the epigenome are attractive therapeutic targets in rheumatic diseases. A key issue is developing agents that target a limited number of disease-related genes and to do this effectively will require a much greater understanding of regulatory pathways. Progress is being made, however, as shown by the development of a small molecule targeting the catalytic domain of an enzyme responsible for the demethylation of a single amino acid in the histone 4 protein. This results in profound anti-inflammatory activities in macrophages [61].

Therapeutic targeting of HDACs

RA

In animal models of RA, HDAC inhibitors (HDACis) have been shown to be effective therapeutic agents. Autoantibody-mediated arthritis is attenuated by a single intravenous infusion of FK228, an inhibitor of HDAC1 and 2, inhibiting of synovial swelling and bone and cartilage loss, reduced TNF and IL-1β production and cell cycle arrest via the up-regulation of p21 [62]. This agent also inhibits *in vitro* proliferation of RASFs [62]. Knockdown of HDAC1 in RASFs by small interfering RNA resulted in decreased proliferation and increased apoptosis [40]. The superior efficacy of MS-275 over other HDACis in collagen-induced arthritis has been proposed to be due to its specificity for class I HDACs, particularly HDAC1 [63]. These data implicate HDAC1 as a key regulator of the autoaggressive phenotype of RASFs. A small study has recently reported that Givinostat, a class I and II HDACi, was both efficacious and safe in a 12-week trial involving 17 patients with systemic-onset JIA [64].

OA

The majority of studies to date have examined the effects of HDACis on chondrocytes and cartilage explants. Inhibition of class I and II HDACs using sodium butyrate or trichostatin A (TSA) resulted in the blocking of pro-inflammatory cytokine–induced cartilage breakdown and suppression of matrix degrading protease production, including MMP-1 and MMP-13 and ADAMTS-4, -5 and -9 [65]. These agents also prevented the IL-1β-induced release of IL-17, TNF, prostaglandin E_2 and NO by chondrocytes [66]. The only published *in vivo* study involved systemic treatment of a rabbit model of OA with TSA, which resulted in reduced cartilage loss and metalloproteinase expression [67].

SLE

Administration of TSA to MRL/lpr mice resulted in reduced expression of IL-6, -10 and -12 and lessening of renal damage and splenic weight [68], and broadly similar results were obtained after administration of this agent to NZB/W F1 mice [69]. Aberrant gene expression is a feature of helper T cells in lupus, including up-regulation of CD40L (CD154) and IL-10, and down-regulation of IFN-γ, these alterations can be reversed by incubation with TSA [70].

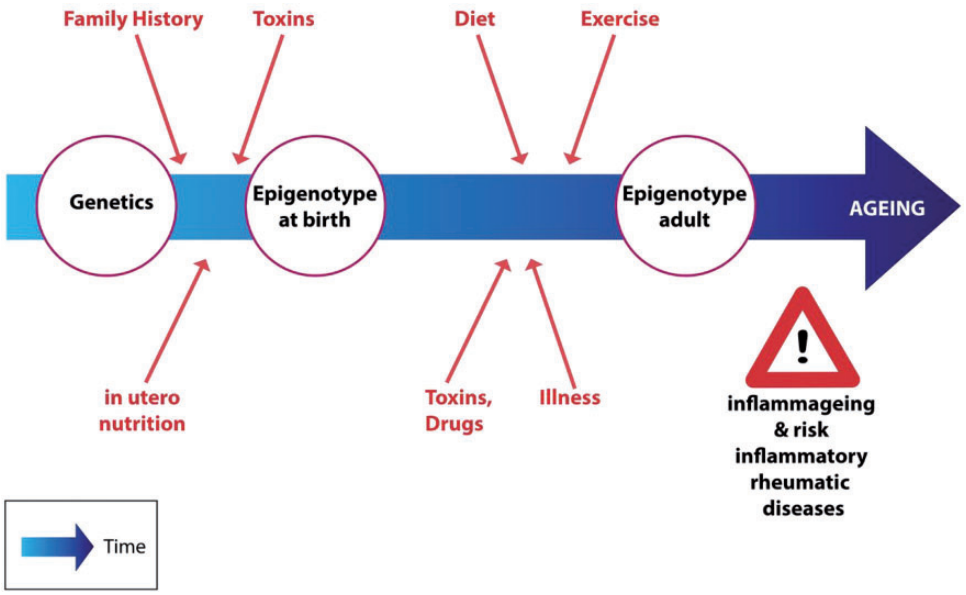
Scleroderma

Transforming growth factor β (TGF-β) controls the production of type 1 collagen synthesis by dermal fibroblasts. This activity can be suppressed by TSA via the down-regulation of Sp1 activity [71] and the inhibition of DNA binding of the profibrotic Smad transcription factors [72]. In addition, TSA inhibits HDAC-7 expression by SSc fibroblasts. Silencing of HDAC-7 expression results in decreased constitutive and cytokine-induced production of collagen [73].

Epigenetics and inflammageing

Ageing is associated with an increased risk of developing a large number of inflammatory rheumatic diseases [74]. Many features of both the adaptive and innate immune systems change with increasing age, leading to a state of increased activity termed inflammageing [75]. In the adaptive system, changes include reduced generation of high-affinity antibodies after immunization [76] and reduction in the naive T cell population [77]. Innate immune system changes include both higher systemic levels of proinflammatory cytokines and increased lipopolysaccharide (LPS)-induced production of TNF and IL-6 by macrophages [78, 79]. Age-related functional changes occur in granulocytes, including decreased chemotaxis, phagocytosis and superoxide generation in response to danger signals [80]. Neutrophils of elderly individuals exhibit decreased expression of CD16 (FcγRIIIa) [81] and have alteration of mitogen-activated protein kinase (MAPK) activation, contributing to the inability of GM-CSF to

FIG. 2 Ageing is associated with epigenetic drift with resultant increased risk of inflammatory conditions.



Environmental exposures during life affect the epigenetic signature of genes resulting in a gradual loss of control of gene expression in different tissues. These changes in immune and inflammatory cells result in the development of inflammageing with increased risk of age-related inflammatory diseases.

decrease caspase-3 activation, leading to reduced clearance of apoptotic neutrophils [82]. These studies suggest a complex pattern of age-related changes in gene expression in different immune cells that may result in an increased risk of inflammatory disorders (Fig. 2).

There is evidence suggesting a role of epigenetic drift in inflammageing. Age-related divergence of the epigenetic signature in peripheral blood has been reported in monozygotic twins; the patterns of global and gene-specific DNA methylation are similar in early life, however, older twins (age >50 years) have marked differences, particularly if they were separated early in life [83]. A longitudinal study of the methylation status of 1505 CpG motifs in 807 genes reported changes in immune genes, including *IL-10* and *IL-16* [84]. Levels of methylation of CpG motifs in the TNF promoter gradually decease with age, by ~1.4% per decade in macrophages, and may be a mechanism of the age-related increased systemic levels of this key proinflammatory cytokine [6]. Inflammation per se can lead to alteration in the epigenetic signature via the effects of reactive radicals oxidizing 5-methylcytosines to 5-hydroxymethlycytosine, with subsequent loss of methylation. Conversely the production of reactive halogen molecules, such as HOCl and HOBr, by activated leucocytes can result in the incorporation of halogen cytosine into DNA with subsequent increased methylation [85].

Epigenome and environmental exposures

The complex relationship between the epigenome and lifestyle factors is emerging. Dietary intake of nutrients, such as folate, is known to affect DNA methylation [86]. Regular exercise has been shown to increase methylation of the *ASC* locus, encoding a component of the NALP3 inflammasome, potentially resulting in reduced levels of proinflammatory cytokines [87]. Cigarette smoking has been linked with alterations in both global DNA methylation and in genes controlling cellular proliferation [88]. An array scan of 14 000 gene promoters reported lower methylation of *F2RL3* [89], intriguingly F2RL2 has been linked with platelet activation and intimal hyperplasia, two of the mechanisms central in the pathogenesis of smoking-related vasculopathy.

Conclusions

There is increasing evidence implicating the epigenome with the development of inflammatory and age-related rheumatic diseases (Table 1). The complexity of the epigenetic signature and its dynamic nature, the differences between cell types and tissues and the potential effects of inflammation on the epigenome complicate studies in rheumatic diseases. Initial attempts have concentrated on candidate genes in specific cell types that are known to be implicated in diseases such as synovial or dermal fibroblasts in RA and SSc, respectively, lymphocytes in SLE and chondrocytes in OA. It is important to note, however, that many of studies have been of low power and have not included a validation study. An additional issue is that many have examined mixed cell populations and it is important to be aware that even a purified cell population such as CD4 T cells includes a heterogeneous mixture of T cell types.

Table 1 Epigenetic alterations in common rheumatic diseases

Disease	Cell type	Epigenetic difference from control	Reference
RA	RASF	↓DNA methylation of cell adhesion and migration genes	[35, 92]
		↑Histone acetylation and HDAC1 expression	[39]
	Peripheral blood mononuclear cells	↓IL-6 methylation	[7]
	CD4 T cells	↓CD40 methylation	[43]
OA	Chondrocytes	↓Leptin, MMP-9, MMP-13, IL-1β and ADMSTS-4 methylation	[45, 46, 93]
SLE	T cells	↓DNA methylation and DNMT1 expression	[41, 53]
SSc	Dermal fibroblast	↑DNA methylation and DNMT1 expression	[58]

Additional issues to be considered are that epigenetic differences may arise secondary to disease or therapies. A recent RA study highlighted novel computational approaches to deal with these confounders [90]. The epigenome is an attractive therapeutic target and *in vitro* and *in vivo* studies with HDACis have shown promising results in rheumatic diseases. However, greater therapeutic specificity is required to minimize side effects, and this will require a greater understanding of the molecular mechanisms controlling the epigenome. Recent studies targeting specific epigenetic marks with small molecules may prove effective [62]. Recent rapid advances in high-throughput technologies and computational biology should ensure that epigenome-wide association studies in cell types implicated in rheumatic diseases will be performed in the near future. This will lead to significant improvements in our understanding of their pathogenesis of these conditions and lead to improved therapeutic strategies [91].

Rheumatology key messages

- Epigenetics factors have been implicated in the pathogenesis of common rheumatic diseases including RA, OA, SLE, and SSc.
- Environmental exposures and ageing are associated with changes in the epigenetic signature and expressed genome.
- Therapeutic targeting of the epigenome has shown efficacy in animal models of RA and preliminary studies in patients.

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